GROWTH OF *ANABAENA* STRAINS (CYANOPHYCEAE) EXPOSED TO CROSSED GRADIENTS OF LIGHT AND TEMPERATURE

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ABSTRACT. — A device to test *Anabaena* strains under a crossed gradient of light intensity and temperature is described. Different strains of one and the same species produced similar growth patterns during crossed gradient culturing. Growth patterns of strains belonging to different species were similar in some strains but different in most strains.

The results are discussed with regards to the taxonomy and ecology of the species investigated.

KEY-WORDS : *Anabaena*, growth, light and temperature, crossed gradient.

INTRODUCTION

General morphology and akinete germination patterns of a number of *Anabaena* strains in culture were described by STULP & STAM (1982). This study led to the conclusion that with those morphological criteria which are traditionally used in *Anabaena* taxonomy, the strains involved could be identified as representatives of six different species described earlier (GEITLER, 1932). Incubation of the strains under different light and temperature conditions hardly affected these taxonomic important morphological characters (STULP, 1982).

The present paper deals with growth of *Anabaena* strains under such conditions with the aid of a so-called light/temperature crossed gradient plate. For establishing growth responses of microalgae, such a plate is a very useful tool. Algae are grown on a surface that is submitted to a gradient of light intensities in one direction and a temperature gradient in a direction perpendicular to the first.

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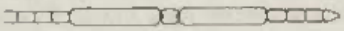
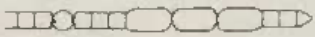
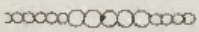
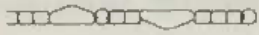
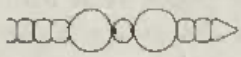
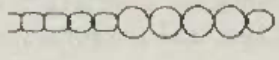
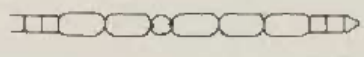
Crossed gradient plates have been described by HALLDAL & FRENCH (1956, 1958) and EDWARDS & VAN BAALEN (1970). They report successful applications : strains of unicellular Cyanophyceae and Chlorophyceae produced reproducible growth patterns. The number of reports about culturing filamentous blue-green algae on crossed gradients is very small. According to HALLDAL et al. (1957) it is very difficult to inoculate gradient plates adequately with these algae . In the present paper modifications of the gradient plate and the method of inoculation are described. The modified gradient plate allowed the establishment growth patterns in *Anabaena*.

Table 1. Main features of the Anabaena strains used

Morphological group	Strain number	Species name	Trichome architecture[a]
1	1609 1611 1403/2a	A. cylindrica	
2	377 1403/4b	A. variabilis	
3	1613 1618 103	A. cf. subtropica	
4	1619	A. cf. verrucosa	
5	1403/13a	A. cf. flos-aquae	
6	1616	A. sphaerica	
7	1823	A. randhawae	
8	106	A. torulosa	

[a] Relatively small cells are vegetative cells, relatively great cells, mostly with deviating forms, are akinetes. Heterocysts are marked with one black spot situated at each cross wall.

MATERIAL AND METHODS

Strains

The majority of the used strains (table 1) have been described before (STULP, 1982). Newly added strains are : strains 1823, 103 and 106. Strain 1823 (obtained from the Culture Collection of Algae at the University of Texas; STARR, 1964, 1978) was originally isolated by VENKATARAMAN (1958) from rain-water puddles in New Delhi (India) and designated as *Anabaena randhawae* spec. nov.

Strains 103 and 106 were isolated by the first two authors from fresh-water habitats in the vicinity of the city of Groningen (The Netherlands). Isolates from these two strains were obtained by means of combinations of general methods like dilution, micro manipulation and spray plating, and those methods particularly developed for motile and akinete producing blue-green algae (VAARA et al., 1979; WIERINGA, 1968). When identified with GEITLER (1932) strain 103 more or less fits in the description of *A. subtropica* Gardner, although small differences in cell dimensions with regard to strains 1613 and 1618 are obvious (table 1, STULP & STAM, 1982). Strain 106 very well fits in the description of *A. torulosa* (Carm.) Lagerh.

Crossed gradient plate

Detailed descriptions of gradient plates are given by HALLDAL & FRENCH (1956) and EDWARDS & VAN BAALEN (1970). In the description of the gradient plate used here special attention will be given to its modifications. Numbers in the description refer to fig. 1.

The algae are grown on a 7 mm high agar surface (800 ml BG-11 medium (RIPPKA et al., 1979) solidified with 2 % agar) in a growth chamber (1) consisting of a glass ground plate (330 x 295 mm) and perspex walls (20 mm high).

The temperature gradient in the agar is obtained as follows : an aluminium plate (2) (400 x 400 x 20 mm) is heated at one end and cooled at the other end by liquids from thermoregulated waterbaths, circulating through holes drilled in the left and right side of the plate. In this way a smooth temperature gradient is obtained in the aluminium plate. As the growth chamber is placed directly on this plate it obtains, by conduction, a temperature gradient as well. The actual temperature at the agar surface can be monitored by means of thermo-couples (3) connected to a recorder (4). The temperature range obtained in this way was from 11°C to 45°C (± 0.1°C).

The light intensity gradient was obtained as follows : two cool white fluorescent light tubes (Philips 40W 33RS) (5) were fitted 110 mm above the backside of the gradient plate. This position results automatically in a decreasing gradient from backside to frontside. This gradient was steepened by placing a sheet of photographic film (6) as large as the culture chamber, and with a continuous gradient from completely translucent at the backside to completely black at the frontside, over the gradient plate lid. The light intensities at the site of the agar surface ranged from 98 $\mu E\ m^{-2}\ s^{-1}$ to 0.6 $\mu E\ m^{-2}\ s^{-1}$ (4500 - 25 lx).

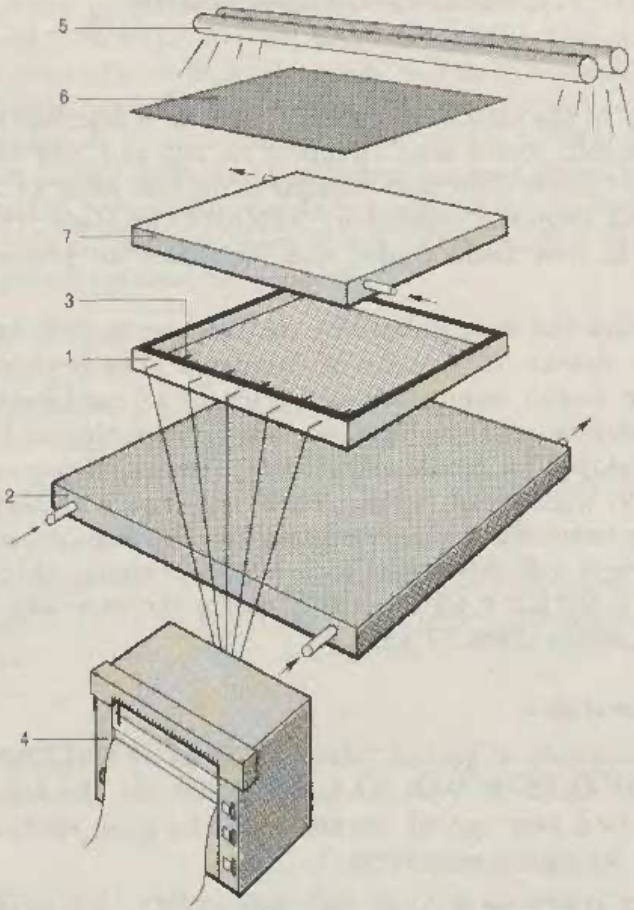


Fig. 1. — Exploded view of the crossed gradient apparatus showing the main parts. 1. Growth chamber, 2. Aluminium plate, 3. Thermocouples, 4. Recorder, 5. Light tubes, 6. Shaded sheat of film, 7. Lid. Small arrows indicate cold liquid circulation, large arrows hot liquid circulation.

To prevent excessive evaporation, which is likely to occur in the high temperature regions of the agar during the time of culturing (the algae were incubated for one week) the growth chamber had to be closed by a lid. This lid consisted of a translucent flat box (7) which was connected to the hot water circuit of the gradient plate. Condensation of water evaporated from the agar on the underside of the lid prevented this way. Foam rubber strips between lid and growth chamber allowed sufficient gas exchange between the growth chamber and the air outside.

Inoculation procedure

The agar surface was inoculated with 2-4 ml of a homogenized fast growing culture by means of a papillary spray (WIEDEMAN et al., 1964). This inoculation method was discussed by HALLDAL & FRENCH (1958) but appeared to be highly successful for *Anabaena* strains.

RESULTS

Light and temperature characteristics of the gradient plate are shown in fig. 2. The temperature gradient is linear. The light intensity is not linear, however the lines connecting spots of equal light intensity are parallel too.

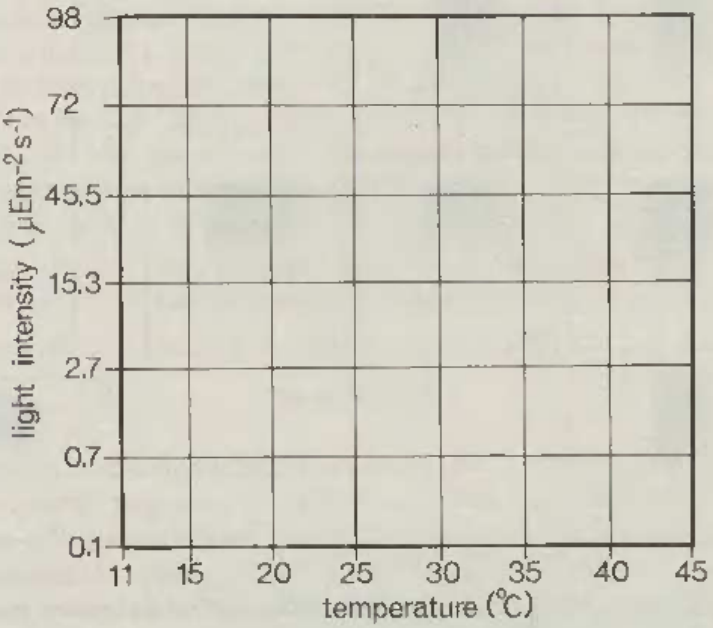


Fig. 2. — Light- and temperature characteristics of the crossed gradient plate.

Fig. 3 shows schematically the growth patterns which were obtained for the tested strains after one week of incubation. The spotted areas represent the light/temperature conditions where growth of the inoculum occurred. After the incubation time there were some disturbances in the agar at the hot end of the plate, in particular around the thermocouples in that area. Since these disturbances — a little shrinking of the agar caused by water evaporation — always arose far outside the growth areas of all tested strains, they could be ignored.

In areas where apparently no growth occurred the condition of the inoculum was not identical. Microscopic observations of the agar surface showed that in high temperature regions no traces of the original inoculum were visible. Evidently the inoculum disappeared by decay, not by active gliding of the trichomes. Control experiments proved that on the relatively high agar concentrations used the mobility of the trichomes of all strains was negligible. In low temperature regions, trichomes of the inoculum were easily detected. They were conserved but simply had not grown out.

The growth patterns shown in fig. 3 are clustered on basis of the micro-

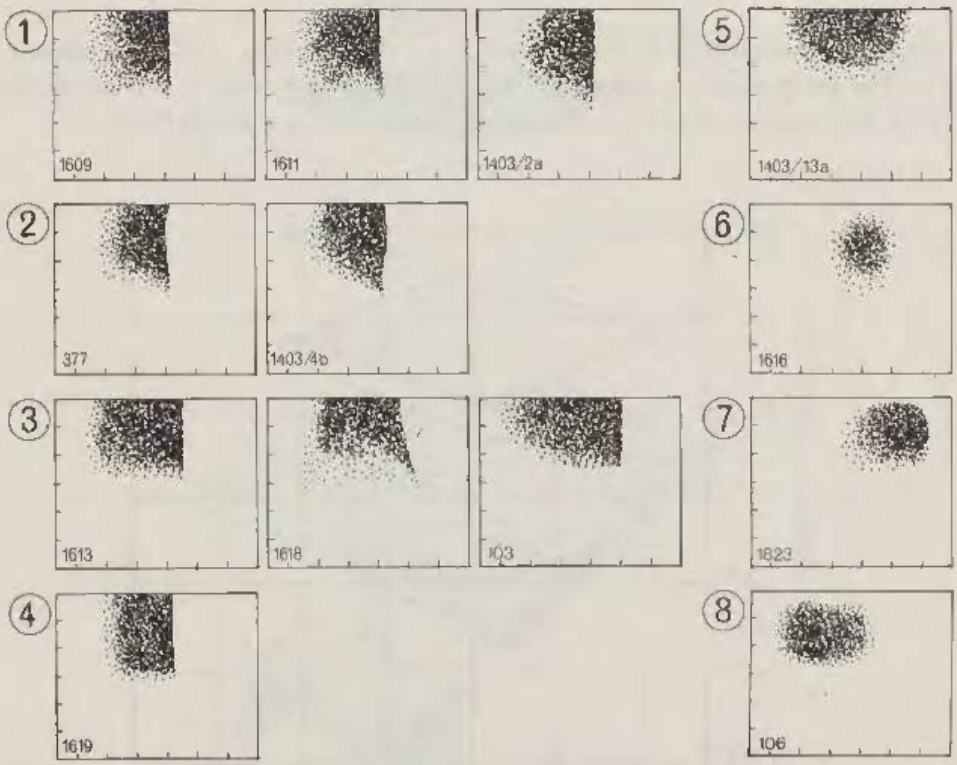


Fig. 3. — Schematic growth patterns of *Anabaena* strains under crossed gradients of light and temperature. For light- and temperature characteristics, see fig. 2. Strain numbers are in small figures (1609, 1611, etc), group numbers (cf. table 1) are in large figures (1-8).

morphological resemblances of the strains (table 1). For all strains only one growth pattern is presented although the strains were tested twice. Differences between the duplicates however were small and could be ignored.

Strains 1609, 1611, 1403/2a, 377, 1403/4b and 1619 (morphological group 1, 2 and 4) showed almost identical growth patterns. Growth occurred in a temperature range of c. 17°C to c. 32°C and a light intensity range of c. 3 to 98 $\mu E\ m^{-2}\ s^{-1}$. A sharp border between growth and no-growth regions was present at tbe upper temperature growth limit. Maximal growth was present at only 1°C below this limit. In regions of low temperature and low light intensities the border was different : growth decreased gradually and there was a wide transition area. At low light intensities strain 1619 had a border parallel to the frontside of tbe gradient plate, whereas strains 1609, 1611, 1403/2a, 377 and 1403/4b had a more or less oblique border. So, these strains were more tolerant to low light intensities at higher than at lower temperatures.

Strains 1613, 1618 and 103 (morphological group 3) showed comparable growth patterns, but they all grew over a wider temperature range. This is illustrated by the location of the upper temperature borders (fig. 3) being

4°C to 8°C higher than those of the strains of morphological group 1, 2 and 4. Strain 103 showed the widest temperature range (c. 16°C to 40°C). The colour of the growth area of strain 1613 differed from all other strains in being dark to almost blackish green. The special colour of this strain in liquid culture has been mentioned previously (STULP & STAM, 1982). Strain 1618 showed oblique borders both at high temperatures and low light intensities.

The growth pattern of strains 1403/13a, 1616 and 106 differ considerably from the general pattern mentioned above. To all sides the growth area had an indistinct fringe (the existence of such a fringe for strain 1403/13a at the upper light intensity border could not be established). The usual sharp high temperature border was lacking. Distinct mutual differences between the strains were : the size of the growth areas, the upper light intensity border and the lower- and upper temperature borders (fig. 3).

Strains 1823 showed an intermediate growth pattern, with a more or less distinct upper temperature- and light intensity border. This strain was able to grow at up to 41°C, a major difference in comparison with all other strains.

DISCUSSION

Application of crossed gradient experiments is a successful and rapid method to establish growth responses of *Anabaena* strains to a combination of varying temperatures at light intensities. Differences in these responses are easily detected by comparing the «light temperature finger-prints» of the strains. The crossed gradient plate proved a reliable device to test the temperature-light responses of a strain in about one week of culturing. During this week the only problem is keeping the agar in the high temperature regions in good condition. Since no extremely thermophilic organisms were involved this problem was of minor importance in the present experiments. Providing the culture chamber with vapourized air like HALLDAL & FRENCH (1958) suggested could therefore be omitted.

Spraying proved to be a very satisfactory way of inoculation. Provided that the inoculum has been homogenized thoroughly before, even very narrow capillaries give good results. Microscopical tests showed that the inoculum was homogeneously sprayed over the agar surface. This success of spraying is in contrast with conclusions drawn by HALLDAL & FRENCH (1958). They state that spraying (and also painting) has proved to be unpractical and prefer to inoculate the agar on the plate with a layer of a homogenized liquid culture of the alga involved. However, when they applied this method for *Anabaena* cultures it was not completely satisfactory. The obtained layer was far too inhomogeneous.

The differences in growth responses of the various *Anabaena* strains are obvious. The most common growth pattern found in seven of the thirteen strains tested, shows a sharp high temperature border and an indistinct low temperature border. The high temperature border is apparently a lethal border whereas the low temperature border is a growth border. This may be illustrated

furthermore by the following accidental observation : one of the gradient plate experiments had to be terminated after two days of incubation. The inoculum on the plate was allowed to grow further under uniform conditions of 23 μE m^{-2} s^{-1} and 26°C. After a few days uniform growth occurred only on that part of the agar plate where temperatutes had remained below the critical upper temperature (e. g. in fig. 3 - 1, 2, 3 and 4 at the left side of the critical temperature line). Obviously only two days of gradient plate incubation kills the inoculum in the high temperature regions whereas it remains alive in the lower ones.

Strains 1403/13a, 1616, 1823 and 106 showed essentially different patterns. The fact that strain 106 was isolated from a water basin densily inhabited by floating water plants could explain this. Strain 1823 was isolated by VENKATARAMAN (1958) from a rain water puddle in New Delhi, India. Presumably this organism is a soil inhabitant. This again would be in agreement with its light requirements. Furthermore the high temperature range for growth of this strain agrees with the temperatures of its original habitat. Since the provenance of strain 1616 is unknown no comparison between the place of isolation and the growth pattern on the gradient plate can be made.

HALLDAL (1958) describes an *Anabaena* strain which has a high temperature lethal boundary of 43°C. However this result hardly can be compared with the present results since Halldal omitted to mention a strain number or a species name of the *Anabaena* he used. Moreover he did not indicate whether he measured the temperature of the agar or the temperature of the aluminium plate.

Mainly based on micromorphological criteria the strains involved in the present study could be separated into eight morphological groups. It was concluded that these groups are recognizable as eight different species (see table 1; STULP, 1982; STULP & STAM, 1982). The differences between the light/temperature fingerprints obtained in the present study do not contradict this conclusion although morphological differences are not always reflected in the fingerprints. *A. cylindrica*, *A. variabilis* and *A.* cf. *verrucosa* strains only show minor differences. *A. cf. subtropica* strains have fingerprints with a wider temperature range, but also show mutual differences. It was discussed previously (see Materials and methods; STULP & STAM, 1982) that mutual differences in morphology between these strains made the conclusion that they are representatives of one and the same species less clear-cut. The fingerprints of the remaining *A.* cf. *flos-aquae*, *A. sphaerica*, *A. randhawae* and *A. torulosa* strains very well correlate with their specific status.

When comparing the present results with those of STULP (1982) one should be aware of the fact that the latter results concerned only the morphology (not the growth) of the strains under different temperature and light conditions in liquid cultures. Nonetheless, the responses of the strains in both types of experiments are in agreement. For instance, in both experiments strain 1403/13a showed a wide and strain 1616 a much narrower temperature range.

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